Executive Summary

The United States Fish and Wildlife Service's (USFWS) National Investigational New Animal Drug Office in Bozeman, MT, conducted a study (AQUIS-01-EFF.2-09) under Study Protocol Number AQUIS-01-EFF to generate efficacy data needed to help obtain U.S. Food and Drug Administration (FDA) approval for the use of AQUI-S® as a fish anesthetic. Study AQUIS-01-EFF.2-09 (conducted May 4, 2004) consisted of four experiments, in each of which three AQUI-S® "treatments" (40, 60, and 80 mg/L) and one Tricaine-S® (MS-222) "control" (80 mg/L) were used to test channel catfish *Ictalurus punctatus* (CCF; test fish) to and from the "handleable" stage of anesthesia. In Experiments 1 (test fish = juvenile) and 2 (adult), fish were tested at a median water temperature of about 20°C. In Experiments 3 (juvenile) and 4 (adult), fish were tested at a median water temperature of about 30°C. In all four experiments, AQUI-S® doses delivered were analytically verified and, overall, were within ± 25% of target doses.

In each experiment, the AQUI-S® treatments and MS-222 control were each administered to 15 test fish. Consequently, 60 test fish were used in each experiment; and a total of 240 test fish were used in the study. Fish were tested individually to handleable (60-min maximum-time limit allowed), and only fish that became handleable within 60 min were tested to recovery (30-min maximum-time limit allowed). All fish, including those that did not become handleable within 60 min, were monitored for survival.

All AQUI-S®-treated fish (overall median length of juveniles = 14.0 cm; overall median length of adults = 33.0 cm) were tested in anesthetic solutions prepared immediately before use. Based on data collected on all AQUI-S®-treated fish tested to handleable, test fish behavior was characterized as normal in 100% of the juvenile fish tested at both 20 and 30°C, 51% of the adult fish tested at 20°C, and 96% of the adult fish tested at 30°C. Abnormal behavior was most frequently characterized as fish becoming slightly agitated upon initial immersion in AQUI-S® test solution. There appeared to be no lasting effects of the AQUI-S® treatments, and no AQUI-S®-treated fish died during the study. Based on data collected on all AQUI-S®-treated fish when tested to recovery from handleable, test fish behavior was characterized as normal in 100% of the fish tested at both 20 and 30°C.

In all experiments, 100% of the 15 test fish in each AQUI-S® treatment group became handleable within 60 min. Based on data collected at 40, 60, and 80 mg/L AQUI-S®, median times for juveniles to become handleable ranged from 1.02 to 2.62 min, and median times for juveniles to recover ranged from 0.83 to 2.15 min. Median times for adults to become handleable ranged from 1.30 to 3.42 min, and median times for adults to recover ranged from 2.23 to 3.12 min. In all four experiments, median time to handleable decreased as AQUI-S® concentration increased, but Kaplan-Meier (K-M) survival analysis confirmed that only in Experiments 2 and 3 did time to handleable decrease significantly as AQUI-S® concentration increased from 40 to 60 to 80 mg/L. No significant trend was observed between median time for fish to recover from

handleable and concentration of AQUI-S® used to anesthetize fish to the handleable stage. Fish tested with 40 or 60 mg/L AQUI-S® or 80 mg/L MS-222 were returned to the reference population. Fish tested with 80 mg/L AQUI-S® were disposed of according to routine Fish Breeders of Idaho, Inc. procedures.

Comparisons within AQUI-S® treatments were made between Experiments 1 and 2 and between Experiments 3 and 4 to investigate possible life-stage effects on times to and from handleable when median water temperature was held constant at either 20 or 30°C. Comparisons of median times to and from handleable indicate that the juveniles become handleable and recover from handleable faster than adults, and K-M analysis of time-to-handleable and time-to-recovery data confirmed significant differences in times to handleable and times to recover from handleable (except at 20°C, there was no significant difference in time for juveniles and adults to recover from handleable at 60 mg/L AQUI-S®).

Within-treatment comparisons were made between Experiments 1 and 3 and between Experiments 2 and 4 to investigate possible water temperature effects on times to and from handleable when life stage was held constant at either juvenile or adult. Comparisons of median times to and from handleable using the K-M analysis revealed that juveniles and adults become handleable significantly faster at the warm water temperature (i.e., about 30°C) and juveniles recover from handleable significantly faster at the warmer water temperature.

Overall, the AQUI-S® efficacy data generated during study AQUIS-01-EFF.2-09 support the following conclusions:

1. AQUI-S® concentrations of 40, 60, and 80 mg/L are efficacious and safe for inducing juvenile and adult channel catfish to handleable at water temperatures of about 20 and 30°C because induction and recovery times are within that of an “ideal” fish anesthetic and mortality is unlikely to occur;

2. Regardless of water temperature, the time required for juvenile and adult channel catfish to become handleable will likely decrease as AQUI-S® concentration is increased from 40 to 60 to 80 mg/L;

3. At a given water temperature and AQUI-S® concentration, juvenile channel catfish will likely become handleable faster than adult channel catfish; and

4. At different water temperatures and a given AQUI-S® concentration, juvenile and adult channel catfish will likely become handleable faster in the warmer water than in the cooler water.

Table 4. Median time to handleable (HT) and recovery from handleable (HRT) for (a) juvenile channel catfish tested at about 20°C, (b) adult channel catfish tested at about 20°C, (c) juvenile channel catfish tested at about 30°C, and (d) adult channel catfish tested at about 30°C.

Anesthetic concentration (mg/L)	Juvenile at about 20°C		Adult at about 20°C		Juvenile at about 30°C		Adult at about 30°C	
	HT (min)	HRT (min)	HT (min)	HRT (min)	HT (min)	HRT (min)	HT (min)	HRT (min)
AQUI-S								
40	2.62	1.43	3.42	2.72	2.13	0.97	2.23	2.33
60	1.78	2.15	2.37	3.12	1.50	0.90	1.65	2.23
80	1.63	1.78	1.80	2.98	1.02	0.83	1.30	2.33
MS-222								
80	2.85	0.87	6.68	1.25	2.62	0.83	4.98	1.22